respectfully requested in light of these amendments and the following remarks.

I. Restriction Requirement

The Examiner has made a Restriction Requirement as follows:

Group I, claims 1-32, drawn to methods for screening nucleic acid duplex stability; and

Group II, claims 33-37, drawn to kits comprising labeled donor and acceptor FET-labeled nucleic acids.

The Examiner suggests that these Groups do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features. Specifically, the Examiner suggests that the claims lack a special technical feature because there are at least two references which the Examiner suggests anticipate claim 1.

Applicants respectfully traverse this Restriction Requirement.

At the outset, it is respectfully pointed out that the Examiner's suggestion that "the inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or

corresponding special technical feature" directly contradicts both the Search Report and the Written Opinion issued by the PCT application of which this case is the U.S. National Stage.

Further, U.S. Patent 5,972,612 was cited in the Search Report as a document defining the general state of the art which is not considered to be of particular relevance. Accordingly, Applicants respectfully disagree with the Examiner's suggestion that this reference anticipates claim 1, particularly since this rejection was not raised anywhere else in the Office Action.

Finally, MPEP §803 provides two criteria which must be met for a restriction requirement to be proper. The first is that the inventions be independent or distinct. The second is that there would be a serious burden on the Examiner if the restriction is not required. A search of the prior art relating to the methods and kits of the present invention was already performed in the PCT application. Further, additional searching of the methods of Group I would reveal any art relating to kits for carrying out such methods. Thus, there is clearly no serious burden placed upon the Examiner by including all claims in this case.

Accordingly, since this Restriction Requirement does not meet both criteria as set forth in MPEP § 803 to be proper, it is

respectfully requested that this Restriction Requirement be withdrawn.

However, in an earnest effort to be completely responsive, Applicants elect Group I, claims 1-32 with traverse.

II. Abstract

The Examiner suggests that the application does not contain an Abstract of the disclosure as required by 37 C.F.R. 1.72(b). It is respectfully pointed out that this application is a National Stage of a PCT application and the Abstract actually was provided on the cover page of the application. However, in an earnest effort to be completely responsive, Applicants are providing herewith an Abstract for insertion as page 63 of the application.

III. Claim Objections

Claims 16-29 are objected to under 37 C.F.R. 1.75(c) as being in improper form as a series of multiple dependent claims depending upon each other. Accordingly, in an earnest effort to advance the prosecution of this case Applicants have amended the claims so that no multiple dependent claims depend from a multiple dependent claim. Withdrawal of this rejection and consideration of these claims is therefore respectfully

requested.

IV. Rejection of Claims 1-17 and 30-32 under 35 U.S.C. § 112, second paragraph

Claims 1-17 and 30-32 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner suggests that clarification of the number of strands in the initial solution and which strand is the target and which strand is the focus of the target strand is necessary.

Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended the claims to clarify that the initial nucleic acid duplex comprises a first nucleic acid strand and a second nucleic acid strand and that the focus of the target strand is to either the second nucleic acid strand of the initial nucleic acid duplex or the first or second nucleic acid strand of the initial nucleic acid duplex. Support for these amendments is provided throughout the specification in both the summary and the detailed description. These amendments clarify the number of strands in the initial solution as well as the focus of the target strand.

Accordingly, withdrawal of this rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

V. Rejection of Claims 1-15 and 30-32 under 35 U.S.C. § 103(a)

Claims 1-15 and 30-32 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Albrecht et al. U.S. Patent 6,625,163 issued July 24, 2001 in view of Drmanac et al. U.S. Patent 5,525,464 issued July 2, 1996.

The Examiner suggests that Albrecht et al. teaches use of a method for competitive hybridization between labeled DNA strands derived from a plurality of cells or tissues. Further, the Examiner suggests that Albrecht et al. teaches that quantification may be obtained from competitive hybridization or an association-disassociation relationship.

The Examiner suggests that Drmanac et al. teaches methods for determining the hybridization stability and its relationship to other thermodynamic properties as related to the function of competitive equilibria and that the stability is related to the uninterrupted Watson-Crick base pairing. In addition, the Examiner suggests that Drmanac teaches the theoretical principles of oligonucleotide hybridization to filter bound target nucleic acids only a few nucleotides longer than the probe in conditions

of probe excess is a pseudo-first order reaction with respect to target concetrations. Further, the Examiner suggests that Drmanac et al. teaches methods by which the concentrations and total volumes of the nucleic acids in each solution are stringently controlled.

In addition, while not cited as a prior art reference in the rejection of claims 1-15 and 30-32 under 35 U.S.C. § 103, the Examiner suggests that Breslauer.(PNAS 1986 83:3746-3750) teaches that given a set of solution conditions, the relative stability of the DNA duplex structure may be predicted such as ΔH°, ΔG°, and ΔS°.

Therefore, the Examiner suggests that it would be *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of measuring duplex stability as taught by Breslauer et al. with the competitive hybridization of Albrecht by measuring the ideal duplex stability of probes. The Examiner suggests that motivation to do so is provided by Breslauer's statement in the Abstract that "this capability would prove valuable in predicting the stability of gene-probe complex, selecting optimal conditions for a hybridization experiment, deciding on the minimum length of a probe, predicting the influence of specific transversion or

transition on the ability of an affect DNA region, and predicting the relative stabilities of local domains with a DNA duplex. Further, the Examiner suggests that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was conceptualized to utilize the temperature dependent melting procedures of well controlled solutions and volumes and the working theoretical principles and procedures of Drmanac in the competitive binding assays of Albrecht to obtain the thermodynamic stability data.

The Examiner also suggests that one of ordinary skill would be motivated to use fluorescent strands for detection of hybridization and mismatches or other anomalies by combining the methods of Albrecht and Drmanac.

Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, three basic criteria must be met. MPEP 2143. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all claim limitations.

The rejected claims of the present application are all inclusive of the step of subjecting the solution of initial nucleic acid duplex and target sequence to conditions which disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first and/or second nucleic acid strand of the initial nucleic acid duplex of step prior to a competitive re-hybridization step of the now disrupted initial nucleic acid duplex and the target strand.

This disruption step is not taught or suggested by either Albrecht et al. or Drmanac et al. Accordingly, these references, alone or in combination fail to teach or suggest all the claim limitations of the pending claims.

Further, Applicants respectfully disagree with the Examiner that one of skill would be motivated to include this disruption step in the competitive hybridization assay taught by Albrecht et al. for use in monitoring differential gene expression by massively parallel analysis for all or a substantial fraction of expressed genes (see col. 2, lines 21-24 of Albrecht et al.) based upon the teachings of Breslauer in Proc. Natl Acad. Sci. USA 1986.

The 1986 reference of Breslauer provides a report of the complete thermodynamic characterization of 10 nearest neighbor

interactions possible in a Watson-Crick DNA duplex structure and a demonstration of how such data can be used to predict the stability and the melting behavior of any DNA duplex from knowledge of its primary sequence. This reference is unrelated to methods for determination of differential gene expression in cells and tissues as taught by Albrecht et al. and methods of sequencing a target nucleic acid as taught by Drmanac. This is clearly evidenced by the fact that neither of these methods is included in the list of applications to which the teachings of Breslauer et al. are taught to be valuable (see Abstract of Breslauer et al.). Accordingly, one of skill in the art would have no motivation to combine the teachings of Breslauer et al. with Albrecht et al. and/or Drmanac.

Thus, the cited combination of prior art fails to meet the criteria required to render the claimed invention *prima facie* obvious.

Withdrawal of this rejection under 35 U.S.C. § 103(a) is therefore respectfully requested.

VI. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly,

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favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please insert the following Abstract at page 63 of the application:

Abstract

Simple methods and kits for determining the thermodynamic stability of nucleic acid duplexes and single polynucleotide polymorphisms via competitive equilibria are provided.

In the Claims:

Please amend the claims as follows:

1. (amended) A method for screening for nucleic acid duplex stability by competitive equilibria comprising:

(a) producing a solution containing a known amount of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to a target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) titrating the solution with a second solution comprising a known concentration of the target nucleic acid strand which

competes with the first nucleic acid strand of the initial nucleic acid duplex of step (a) for binding to the second nucleic acid strand of the initial nucleic acid duplex of step (a), said target nucleic acid strand being single- or double-stranded;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the target strand and the second nucleic acid strand of the initial nucleic acid duplex of step (a) upon titration in step (b), but which do not disrupt the target strand when double-stranded;

(d) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(e) monitoring the titrated solution for changes in the amount of initial nucleic acid duplex formed as a function of the amount of target nucleic acid strand added.

2. (amended) The method of claim 1 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the target strand and the second nucleic acid strand of the initial nucleic acid duplex of step (a) upon titration in step (b), but which do not disrupt the target strand when double-stranded and

the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

3. (amended) A method for screening for nucleic acid duplex stability comprising:

(a) producing a solution containing an initial nucleic acid duplex ~~comprising an initial nucleic acid duplex~~ with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand and a second nucleic acid strand, each strand being capable of forming a duplex with a double-stranded target strand;

(b) titrating the double-stranded target strand into the solution ~~to compete with initial nucleic acid duplex formation by forming duplexes of target strand and first nucleic acid strand and target strand and second nucleic acid strand~~;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target strand, and any duplex between the disrupted target strands and the first and second nucleic acid strands of the initial nucleic acid duplex of step (a);

(d) subjecting the titrated solution to conditions which promote duplex formation; and

(e) monitoring the titrated solution for changes in the

amount of initial nucleic acid duplex formed as a function of the amount of double-stranded target nucleic acid strand added.

4. (amended) The method of claim 3 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a), the double-stranded target duplex of step (b) and any duplexes formed between the disrupted target strands and the first or second nucleic acid strands of the initial nucleic acid duplex of step (a) upon titration in step (b) and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

5. (amended) A method for extracting enthalpy data from the competitive equilibria method of claim 2 ~~or 4~~ comprising controlling temperature during step (d) so that changes monitored in step (e) can be collected as a function of temperature to produce a family of titration curves that can be used to extract enthalpy (ΔH°) data.

6. (amended) A method for detecting a single nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the initial nucleic acid duplex is designed to identify

a single nucleotide polymorphism in a single- or double-stranded target nucleic acid sequence;

(b) measuring the amount of the initial nucleic acid duplex produced in step (a);

(c) adding a fixed excess amount of the single- or double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the single- or double-stranded target strand and the first or second nucleic acid strand of the initial nucleic acid duplex of step (a) upon addition of the single- or double-stranded target strand in step (c), but which do not disrupt the target strand when double-stranded;

(e) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(f) measuring the amount of initial nucleic acid duplex formed after addition of the single- or double-stranded target strand wherein the measured amount after addition of the single- or double-stranded target strand is indicative of the single- or double-stranded target strand containing the single nucleotide polymorphism.

7. (Amended) The method of claim 6 wherein the conditions

in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the single- or double-stranded target strand and the first or second nucleic acid strand of step (a) upon addition of the single- or double-stranded target strand in step (c), but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

8. (amended) A method for detecting a single nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the duplex is designed to identify a single nucleotide polymorphisms in a double-stranded target nucleic acid sequence;

(b) measuring the amount of the initial nucleic acid duplex;

(c) adding a fixed excess amount of a the double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target nucleic acid sequence and any duplex formed between the double-stranded target strand and the first or second nucleic

acid strand of the initial nucleic acid duplex of step (a) formed upon addition of the double-stranded target strand in step (c);

(e) subjecting the titrated solution to conditions which promote duplex formation; and

(f) measuring the amount of initial duplex formed after addition of the target strand wherein the measured amount after addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism.

9. (amended) The method of claim 8 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex of step (a), the double-stranded target duplex and any duplexes formed between the disrupted target strands and the first or second nucleic acid strands of the initial nucleic acid duplex of step (a) upon addition of the double-stranded target duplex in step (c) and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

10. (amended) The method of claims 6 ~~through 9~~ wherein one nucleic acid strand of the duplex formed in step (a) contains a sequence corresponding to the targeted single nucleotide polymorphism; and the measured amount of initial duplex formed

after addition of the target strand indicative of the target strand containing the single nucleotide polymorphism in step (f) decreases as compared to the amount measured in step (b).

11. (amended) The method of claims 6 ~~through 9~~ wherein one nucleic acid strand of the duplex formed in step (a) is a wild type sequence; and the measured amount of initial duplex formed after addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism in step (f) is approximately equal to the amount measured in step (b).

12. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) adding a known volume and concentration of an initial nucleic acid duplex with a known stability to a known volume of a solution containing a target strand, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex between the target strand and a the first nucleic acid strand or the second nucleic acid strand of the initial nucleic acid duplex of

step (a);

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution.

13. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) adding a known volume of a solution of target strand to a known volume of a solution containing a known concentration of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex between the target strand and a the first or second nucleic acid strand of the initial nucleic acid duplex;

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution.

14. (amended) The method of claim 12 ~~or 13~~ wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strand of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.

15. (amended) A method for assessing stability of various selected target strands comprising:

(a) selecting various target strands;

(b) performing the method of claim 1 with the same initial nucleic acid duplex and each of the selected target strands; and

(c) comparing monitored changes in the amount of initial nucleic acid duplex formed as a function of the amount of the selected target nucleic acid strand added to ascertain differences in stability of duplexes or triplexes formed by the various target strands.

25. (amended) The method of any of claims 1 through ~~24~~ 15 or 38 through 45 wherein at least one nucleic acid strand of the initial duplex comprises an internal loop, a modified base, a

modified backbone, or a non-Watson-Crick nucleotide base variation.

28. (amended) The method of claims 1 through ~~27~~ 15 or 38 through 45 wherein at least one nucleic acid strand of the initial nucleic acid duplex is immobilized to a solid support.

30. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) measuring fluorescence of a known volume of a solution containing a single- or double-stranded target nucleic acid sequence;

(b) adding a known volume and concentration of an initial nucleic acid duplex to the solution, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target nucleic acid sequence and a second nucleic acid strand having a sequence wholly or in part complementary to the target nucleic acid sequence;

(c) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strands of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded;

(d) subjecting the solution to conditions which promote

duplex or triplex formation; and

(e) measuring the fluorescence of the solution after step (d) so that a relative change in the fluorescence can be determined.

31. (amended) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) measuring fluorescence of a solution containing a known volume and concentration of an initial nucleic acid duplex, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand;

(b) adding a known volume of a single- or double-stranded target nucleic acid sequence to the solution;

(c) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strands of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded;

(d) subjecting the solution to conditions which promote duplex or triplex formation; and

(e) measuring the fluorescence of the solution after step

(d) so that a relative change in the fluorescence can be determined.

32. (amended) The method of claims 30 or 31 wherein the conditions in step (c) comprise heating the titrated solution to a temperature high enough to disrupt the initial nucleic acid duplex and any duplex or triplex formed between the target strand and the first or second nucleic acid strand of the initial nucleic acid duplex, but which do not disrupt the target strand when double-stranded and the conditions of step (d) comprise cooling the titrated solution to a temperature wherein duplex formation occurs.